

27821 Course Schedule in short

In general: We will be running a total of 5 biofilm/flow cell experiments (ex 1- 5). Each team will be responsible for one flow cell system. This means that the team must assemble the system, sterilize it, and fill it with medium according to the instructions. Furthermore, the team must daily check that the medium supply is sufficient and empty the waste. All teams will inoculate and work with flow channels from all of the six systems, so the “responsibility” is limited to the above mentioned maintenance tasks. Teams that are going to inoculate strains in the free exercise biofilm system will also be responsible for media supply and waste in this system.

There is in addition one adhesion experiment and one diagnostic exercise.

The free exercise is what the name implies: free. This means you can decide which experiments you want to perform and which analyses you want to do. The work on this exercise is administered almost entirely by yourself (there are, however, some time slots assigned to this). *Bring your own samples of interest for the free exercise or sample from the local area during the first part of the course.*

The following schedule is a guideline. Changes may occur. Changes are announced at the morning gatherings at 9:00

Friday 23/8 (Copenhagen University):

9:00 The exercises and practical information

9:10 Talk: Susanne Knøchel: Reemergence of bacterial contamination in food plants – are biofilms to blame?

Technical Talk: Janus Haagenzen and Claus Sternberg: Biofilms and tools, confocal microscopy.

10:30 Coffee Break

10:45 Henrik Sigumfeldt: Make groups and Intro to Adhesion exercise.

11:15 Tour of the institute

11:45 Lunch.

12:45 Adhesion assay.

5:00 End of day 1

Saturday 24/8 (DTU):

9:00 Video: Assembling flow systems and intro to BF system 1-3 (Janus Haagenzen)

10:00 Building Biofilm setups (All in all we will work on 5 systems during the course, each team of 3 will be responsible for 1 system with respect to media preparation and waste removal, all teams will work on all systems)

Medium preparation for all biofilm systems.

12:00 Lunch.

1:00 Sterilization of biofilm system2, and 4/5.

The free exercise: Sampling of bacteria from your own chosen environment (contact lenses, soil, plants, foods items etc). Isolation of bacteria/plating and incubation at different temperatures.

3:00 Inoculation of already prepared and sterilized system 1 and 3.

5:00 Rounding up in the lab with things you did not manage during the day
End of day 2

Sunday 25/8 (DTU):

9:00 Lea Madsen: Intro to the diagnostic exercise

Microtiter assay: inoculation of 2 plates, one that you will proceed with by hand and one that will be handled using a robot (*E. coli* and mutants, each team make their own plates). Incubate until Tuesday.

The free exercise: Inspect plates and continue as you want from own ideas or guidelines in the manual material.

MIC determinations of *P. aeruginosa* determined by different conc. of colistin in test tubes and using E-test (has to be used later in the course for biofilm experiments (system 3)), Confocal microscopy and image acquisition of the *E. coli* strains (biofilm system 1). Will take place most of the day one team at the time (images for COMSTAT).

Start of diagnostic exercise (Multiplex PCR and plate prep)

Microtiter assay: Washing Crystal violet staining as well as introduction to and starting up of the robot with your second microtiter plate (robot operated by the instructors).

12:00 Lunch.

1:00 Technical Talk: Claus Sternberg: COMSTAT

1:30 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).

Diag. exercise contd.

Look at MIC determinations tubes and plates.

Free exercise (continued... QS, make plates for motility assay, biofilm assays, microscopy/morphology structures on plates ...).

6:00 End of day 3.

Monday 26/8 (DTU):

9:00 Talk: Søren Molin: title tba

9:45 Talk: Mette Burmølle: Use of Bioflux for biofilm studies

10:10 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).

COMSTAT on obtained images

Imaris treatment of images.

Inoculation of biofilm system2, and 4/5 (if you have isolated strains you would like to see develop in a flow cell).

Diagnostic exercise: Antibiotic test. Hybridization/detection

Free exercise

12:00 Lunch.

1:00 Talk: Lars Jelsbak: Bacterial evolution in chronic infections

2:00 Addition of antibiotics to biofilm system 3.

Diag. exercise contd.

Analyzing data from COMSTAT.

Work on the free exercise (continued...).

5:00 End of day 4.

Tuesday 27/8 (DTU):

- 8:30** Staining of biofilm system 3 with Propidium Iodid (*P. aeruginosa* +/- antibiotics and *P. aeruginosa* (Yfp)+ *P. aeruginosa* pilA (Cfp)) (only team 3)
- 9:00** **Talk: Anders Folkesson: Biofilm formation and antibiotic tolerance: is there a connection?**
- 10:00** **Technical Talk: Claus Sternberg: FISH**
- 10:20 Diagnostic exercise: Inspect plates. Data analysis.
- 11:00 Image acquisition of biofilms, system 3 continued during most of the day
Inspect biofilm system 2 before FISH plating and sorting tomorrow.
Read micro titer assay (BF 1) on Elisa reader
- 12:00 Lunch.
- 1:00** **Talk: Per Væggemose: Hygiene and biofilms – a problem in food industry.**
- 2:00 Image acquisition of biofilms, system 3 continued during most of the day
Diagnostic exercise contd.
Work on your free exercise (follow biofilm development if you have inoculated flow system 5 with your isolated strains)
Inspect biofilm system 2 before FISH, plating and sorting tomorrow. Treat images using Imaris, COMSTAT.
Lottery for student presentations.
- 6:00 End of day 5

Wednesday 28/8 (DTU):

- 9:00** **Lars Behrend, Microenvironmental analysis of biofilms – techniques and concepts**
- 10:00 Embedding and hybridization+harvesting and fixation and plating of biofilms (*P. aeruginosa* + *Acinetobacter*; biofilm system 2) (procedure continues throughout the whole day).
Work on the free exercise (continued...). Diagnostic exercise: finishing.
- 12:00 Lunch (Some teams may have to postpone lunch until embedding has been done).
- 1:00 Continue the work from biofilm system 2
Hybridization contd.
Work on the free exercise (image acquisition of system 4/5, etc).
- 6:00 End of day 6
- 7:30 Dinner (time and place tbd)

Thursday 29/8 (DTU):

- 9:00** **Talk: Morten Alhede, Quorum Sensing and QS inhibition**
- 10:00 Image hybridized samples from biofilm system 2 (Leica microscope)
Image acquisition of biofilm system 4/5 (Zeiss microscope)
- 12:00 Lunch.
- 1:00 Image acquisition of biofilm system 4/5
Work on free exercise Data treatment, preparation of presentation. Inspect the last samples.
Treat images using Imaris.
Cleaning procedures for biofilm systems.
- 6:00 End of day 7.

Friday 30/8 (Copenhagen University or DTU, tba):

- 9:00 The last preparations for the presentations
- 10:30 Presentation of results.
- 12:00 Lunch
- 1:00 Last presentations of results and evaluation.
- 3:00 End of course.

Biofilm Systems used.

Biofilm system 1: *E. coli* (traA), *E. coli* (F+), *E. coli* (traD) mutant, Image acquisition and COMSTAT (15 channel system, Media FB with Fe-EDTA+A-10. Addition of glucose+proline+thiamine).

Biofilm system 2: *P. aeruginosa* (CY5 blue probe) + *Acinetobacter* (Cy3 red probe): FISH, directly on biofilm and syto staining of harvested cells. Also plating of harvested cells on selective plates (PA will grow on 100 µg/ml Amp) (ACN will grow on 100µg/ml Strep) + FACS sorting/counting for estimation of ratio between the species after biofilm establishment harvesting and FISH. (15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 3: *P. aeruginosa* (Gfp) alone two channels +/- antibiotic treatment and *P. aeruginosa* (Yfp) + *P. aeruginosa* pilA (Cfp) for structure development, image acquisition after antibiotic treatment. (15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 4 and 5: Prepared for the free exercise (Participants own strains or strains isolated during the course). We will consider mediums dependent on the strains.

Time lapse will be shown as demonstration videos and explained during the CLSM sessions.